

# Arterial pressure oscillations are not associated with muscle sympathetic nerve activity in individuals exposed to central hypovolaemia

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**Non-technical summary** The body adjusts the level of sympathetic nervous system activation to regulate blood pressure in health and disease. Therefore, knowing the level of sympathetic activation is important to treat patients with abnormal sympathetic nervous system function. Directly measuring sympathetic activation is difficult, however, and not possible in most clinical settings. Previous investigators have shown that the variability in blood pressure increases during sympathetic activation. To determine if this is a good non-invasive index of sympathetic activation, we compared blood pressure variability to the direct measurement of sympathetic activation in healthy humans. We activated the sympathetic nervous system by redistributing blood volume away from the head and brain, which also occurs with prolonged standing or haemorrhage. We show that the relationship between variability in blood pressure and sympathetic activation is not the same in all individuals, indicating that blood pressure variability cannot be reliably used to measure sympathetic activation.

**Abstract** The spectral power of low frequency oscillations of systolic arterial pressure ( $SAP_{LF}$ ) has been used as a non-invasive surrogate of muscle sympathetic nerve activity (MSNA) in both experimental and clinical situations. For  $SAP_{LF}$  to be used in this way, a relationship must exist between  $SAP_{LF}$  and MSNA within individuals during sympathetic activation. Using progressive central hypovolaemia to induce sympathetic activation, we hypothesised that  $SAP_{LF}$  would correlate with MSNA in all subjects. ECG, beat-by-beat arterial pressure and MSNA were recorded in humans ( $n = 20$ ) during a progressive lower body negative pressure (LBNP) protocol designed to cause presyncope in all subjects. Arterial pressure oscillations were assessed in the low frequency (LF; 0.04–0.15 Hz) domain using a Fourier transform. For the entire group,  $SAP_{LF}$ , MSNA burst frequency, and total MSNA increased during LBNP. Values for coefficients of determination ( $r^2$ ) describing the linear associations of  $SAP_{LF}$  with MSNA burst frequency and total MSNA were 0.73 and 0.84, but rose to 0.89 and 0.98 when curvilinear fits were used, indicating that the relationship is curvilinear rather than linear. Associations between  $SAP_{LF}$  and MSNA within each individual subject, however, varied widely for both MSNA burst frequency and total MSNA, whether derived by linear ( $r^2$  range,  $1.7 \times 10^{-6}$  to 0.99) or polynomial ( $r^2$  range, 0.09 to 1.0) regression analysis. Similar results were obtained when relationships between low frequency oscillations in diastolic arterial pressure and MSNA were evaluated. These results do not support the use of low frequency oscillations in arterial pressure as a non-invasive measure of sympathetic outflow for individual subjects during sympathetic activation.

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**Abbreviations** DAP, diastolic arterial pressure; DAP<sub>HF</sub>, high frequency oscillations of diastolic arterial pressure; DAP<sub>LF</sub>, low frequency oscillations of diastolic arterial pressure; HF, high frequency; HR, heart rate; LBNP, lower body negative pressure; LF, low frequency; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; MSNA<sub>LF</sub>, low frequency oscillations of muscle sympathetic nerve activity; RR, respiratory rate; SAP, systolic arterial pressure; SAP<sub>HF</sub>, high frequency oscillations of systolic arterial pressure; SAP<sub>LF</sub>, low frequency oscillations of systolic arterial pressure; SV, stroke volume.

## Introduction

Using haemorrhage as a stimulus, Guyton and Harris demonstrated in 1951 that profound sympathetic activation elicited increases in oscillations of the blood pressure waveform in dogs (Guyton & Harris, 1951). Because these oscillations were attenuated by sino-aortic denervation, the authors suggested that the oscillations were dependent on baroreflex activation of the sympathetic nervous system. Reports later emerged confirming that sympathetic activation induced by a variety of experimental perturbations was associated with increases in the amplitude of low frequency blood pressure oscillations in humans, while perturbations producing decreases in sympathetic activation decreased the amplitude of these oscillations (Malliani *et al.* 1991; Julien, 2006). These so-called Mayer waves occur at a frequency of about 0.1 Hz and may be produced by either the oscillatory activity of centrally located pacemakers controlling sympathetic nerve function or by a resonance within the baroreflex loop (Julien, 2006).

The application of power spectral techniques to interrogate physiological oscillatory patterns led to the quantification of the amplitude of these Mayer waves as systolic arterial pressure oscillations in the low frequency range (SAP<sub>LF</sub>; 0.04–0.15 Hz). The hypothesis that SAP<sub>LF</sub> could be used as a non-invasive surrogate for sympathetic vasomotor tone and nerve activation was first advanced by Pagani *et al.* (1986), who showed that SAP<sub>LF</sub> increased during head-up tilt in humans. SAP<sub>LF</sub> was subsequently shown to be increased during mental stress (Pagani *et al.* 1989) and daytime ambulatory activity (Furlan *et al.* 1990), both states characterised by sympathetic activation. While direct measurement of sympathetic activity was not performed in these early studies, linear relationships between SAP<sub>LF</sub> and muscle sympathetic nerve activity (MSNA) were later identified in human subjects during sympathetic activation induced by sodium nitroprusside infusion (Pagani *et al.* 1997) and head-up tilt (Furlan *et al.* 2000). On the basis of such observations, SAP<sub>LF</sub> has been used as a non-invasive metric for the determination of autonomic abnormalities in disease states such as hypertension (Lucini *et al.* 2002) and Type 1 diabetes (Lucini *et al.* 2009).

However, this viewpoint has been controversial (Parati *et al.* 1995, 2006; Malpas, 1998; Taylor & Studinger, 2006). Taylor *et al.* (1998) demonstrated in humans that resting levels of sympathetic activation vary across individuals of different ages and sexes, and that SAP<sub>LF</sub> does not correlate with these differences. Furthermore, results from animal studies suggest that SAP<sub>LF</sub> is more closely related to low frequency oscillatory patterns in sympathetic nerve activity (i.e. MSNA<sub>LF</sub>) than to the absolute level of sympathetic nerve activation (Persson *et al.* 1992; Stauss *et al.* 1995). Sympathetic activation is also not accompanied by an increase in SAP<sub>LF</sub> under all physiological (e.g. heat stress: Cui *et al.* 2004) or pathophysiological (e.g. congestive heart failure: Radaelli *et al.* 1999) conditions.

Over the past five years, we have created a large database of human experiments (>170) using a protocol of lower body negative pressure (LBNP) that produces central hypovolaemia to the point of haemodynamic decompensation (i.e. presyncope). In the course of this work, we have performed microneurography in a subset of subjects to directly measure MSNA while simultaneously measuring beat-to-beat continuous blood pressure. Because these experiments uniquely provide multiple levels of MSNA in the same subject, we therefore used these data to test the hypothesis that SAP<sub>LF</sub> directly tracks increases in MSNA and MSNA<sub>LF</sub> in all individuals during sympathetic activation induced by progressive central hypovolaemia.

## Methods

### Ethical approval

This study was conducted under a protocol reviewed and approved by the Brooke Army Medical Center Institutional Review Board and the US Army Medical Research and Materiel Command Institutional Review Board, and was conducted at the US Army Institute of Surgical Research, Fort Sam Houston, TX in accordance with the approved protocol and with the tenets of the *Declaration of Helsinki*. Subjects signed a written informed consent document before experimentation.

## Subjects

MSNA recordings were maintained throughout LBNP to either the point of presyncope or to a high ( $-80$  mmHg) LBNP level without a shift in baseline (thereby ensuring that electrode position had not changed) in 20 subjects (16 males and 4 females; age  $30 \pm 9$  years; height  $176 \pm 10$  cm; weight  $81 \pm 11$  kg; means  $\pm$  standard deviation). We have previously used data from subsets of these subjects to investigate relationships between MSNA and heart rate variability metrics during LBNP (Cooke *et al.* 2008) and to understand MSNA responses at the onset of presyncope (Cooke *et al.* 2009). All subjects first completed a medical history and physical examination by a physician to ensure that they had no previous or current medical conditions that would preclude their participation. Female subjects were not pregnant, as confirmed by a urine test performed within 24 h before experimentation. Subjects maintained their normal sleep patterns, refrained from exercise, and abstained from caffeine and other autonomic stimulants at least 24 h before the experiment. They received a verbal briefing along with written descriptions of all procedures and risks associated with the study, and were made familiar with the laboratory, the experimental protocol and all procedures. Subjects were encouraged to ask questions of the investigators and then signed an informed consent form.

## Experimental protocol

Subjects were instrumented with a standard four-lead ECG to record cardiac electrical potentials, and an inflatable finger cuff to record beat-by-beat finger arterial pressure (Finometer Blood Pressure Monitor; TNO-TPD Biomedical Instrumentation, Amsterdam, The Netherlands). End-tidal  $\text{CO}_2$  was monitored on a breath-by-breath basis as subjects exhaled into a face mask (BCI Capnocheck Plus, Smiths Medical, Waukesha, WI, USA), and was used to derive respiratory rate. Multifibre recordings of MSNA were made directly from the peroneal nerve using the microneurography technique (Hagbarth & Vallbo, 1968). Nerve signals were band-pass filtered (100–2000 Hz) and integrated (time constant, 0.1 s) to obtain mean voltage neurograms.

Central hypovolaemia was induced by application of LBNP (Cooke *et al.* 2004). Subjects were positioned supine within an airtight chamber that was sealed at the level of the iliac crest by way of a neoprene skirt. Each subject was brought to the point of presyncope using progressive LBNP. The LBNP protocol consisted of a 5 min control period (baseline), followed by 5 min of chamber decompression at  $-15$ ,  $-30$ ,  $-45$  and  $-60$  mmHg, then additional increments of  $-10$  mmHg every 5 min until the onset of haemodynamic decompensation (i.e. presyncope). Haemodynamic decompensation was identified

by the attending investigator by a precipitous fall in systolic pressure greater than 15 mmHg, progressive diminution of systolic pressure to less than 70 mmHg, bradycardia, and/or voluntary subject termination caused by symptoms such as grey-out (loss of colour vision), tunnel vision, sweating, nausea, or dizziness. All experiments were conducted at room temperature ( $21.7$ – $24.4^\circ\text{C}$ ) and ambient temperature did not change during the experiment ( $23.05 \pm 0.19^\circ\text{C}$  at baseline,  $23.09 \pm 0.17^\circ\text{C}$  at recovery;  $P = 0.85$ ).

## Data analysis

Data were sampled at 500 Hz and digitized to computer (WINDAQ; Dataq Instruments, Akron, OH, USA). Using commercially available data analysis software (WinCPRS, Absolute Aliens, Turku, Finland), R-waves from the ECG as well as systolic (SAP) and diastolic (DAP) arterial pressures generated from the Finometer were detected. Mean arterial pressure (MAP) was calculated as the area under the arterial pressure waveform. Stroke volumes were estimated from the Finometer using the pulse contour method (Jansen *et al.* 1990). The software also detected bursts of MSNA based on two primary criteria: (1) pulse synchronous spontaneous bursts with signal-to-noise ratios of approximately 3:1, and (2) reflex latencies from preceding R-waves of about 1.3 s (Fagius & Wallin, 1980). Burst detection results were then manually edited by a single experienced microneurographer. As the amplitude and area of sympathetic bursts varies among subjects due to electrode position, MSNA was normalized by dividing the integral of all bursts by the number of bursts occurring during the baseline period. Subsequent burst areas were then divided by this number (i.e. burst areas that were equal to the average baseline area were assigned a value of 1.0) and then multiplied by the number of bursts occurring during a given time period for calculation of total MSNA during LBNP (Cooke *et al.* 2009). MSNA is reported as both burst frequency (bursts  $\text{min}^{-1}$ ) and total MSNA (arbitrary units).

Data for all parameters were averaged across the last 3 min of each LBNP stage. If a subject reached presyncope before collection of 3 min of data at a particular LBNP level, data for that level were not used; if a subject reached presyncope after collection of at least 3 min of data, data for that LBNP level were used. To quantify oscillatory rhythms, non-equidistant beat-to-beat data were first interpolated linearly and resampled at a frequency of 5 Hz. Data were then passed through a low-pass impulse response filter with a cut-off frequency of 0.5 Hz. Three-minute data sets were fast Fourier transformed with a Hanning window to obtain power spectra. Spectral power was expressed as the integrated area within the low-frequency (LF =  $0.04 - 0.15$  Hz)

**Table 1. Haemodynamic and oscillatory responses to LBNP**

Variable	Baseline (n = 20)	LBNP 15 (n = 20)	LBNP 30 (n = 20)	LBNP 45 (n = 18)	LBNP 60 (n = 17)	LBNP 70 (n = 13)	LBNP 80 (n = 7)	Recovery (n = 20)
HR (bpm)	63 ± 2	64 ± 2	69 ± 3	76 ± 3*	90 ± 5*	97 ± 5*	102 ± 8*	59 ± 2
SAP (mmHg)	128 ± 2	126 ± 3	122 ± 3*	120 ± 3*	115 ± 3*	110 ± 5*	114 ± 5*	132 ± 3*
DAP (mmHg)	75 ± 2	75 ± 2	75 ± 2	77 ± 2	77 ± 2	76 ± 3	81 ± 3	81 ± 2*
MAP (mmHg)	94 ± 2	93 ± 2	91 ± 2	92 ± 2	90 ± 2*	88 ± 3*	92 ± 3*	101 ± 2*
SV (ml)	96 ± 3	91 ± 3	79 ± 3*	68 ± 3*	55 ± 3*	45 ± 3*	37 ± 5*	97 ± 4
RR (breaths min <sup>-1</sup> )	15 ± 1	14 ± 1	13 ± 1	13 ± 1	13 ± 1	13 ± 1	13 ± 2	15 ± 1
SAP <sub>HF</sub> (mmHg <sup>2</sup> )	2.4 ± 0.3	2.3 ± 0.3	2.4 ± 0.3	2.6 ± 0.3	3.7 ± 0.6	4.3 ± 0.7*	5.3 ± 1.7*	2.0 ± 0.3
DAP <sub>HF</sub> (mmHg <sup>2</sup> )	0.78 ± 0.12	0.84 ± 0.16	0.97 ± 0.19	1.11 ± 0.18	2.00 ± 0.46*	2.14 ± 0.44*	2.25 ± 0.72*	1.56 ± 0.39
DAP <sub>LF</sub> (mmHg <sup>2</sup> )	3.7 ± 0.5	5.6 ± 0.8	6.5 ± 0.9	7.7 ± 1.1*	12.1 ± 1.6*	17.1 ± 2.5*	24.9 ± 2.6*	4.1 ± 0.9
SAP-MSNA coherence	0.46 ± 0.03	0.50 ± 0.02	0.50 ± 0.04	0.59 ± 0.04	0.60 ± 0.05	0.49 ± 0.05	0.66 ± 0.04*	0.42 ± 0.03
DAP-MSNA coherence	0.47 ± 0.03	0.52 ± 0.03	0.52 ± 0.04	0.62 ± 0.04*	0.60 ± 0.05	0.52 ± 0.05	0.68 ± 0.04*	0.44 ± 0.04

HR, heart rate; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; SV, stroke volume; RR, respiratory rate; SAP<sub>HF</sub> and DAP<sub>HF</sub>, spectral power of high frequency (0.15–0.4 Hz) oscillations in systolic and diastolic arterial pressure; DAP<sub>LF</sub>, spectral power of low frequency (0.04–0.15 Hz) oscillations in diastolic arterial pressure. \* $P \leq 0.045$  compared with baseline value.

and high-frequency (HF = 0.15–0.4 Hz) ranges for arterial pressure and MSNA oscillations (Task Force of the European Society of Cardiology, 1996); individually identified burst areas were used for MSNA oscillation determination. We calculated coherence by dividing the cross-spectral densities of the two signals of interest (either SAP or DAP with MSNA) by the product of the individual autospectra, and then averaged coherence within the LF range (deBoer *et al.* 1987).

### Statistical analysis

Analysis was accomplished using commercially available software (SigmaStat; Systat Software, San Jose, CA, USA). A one-way (LBNP level) randomized block (subjects) ANOVA for repeated measures was used for comparison of outcome variables across LBNP level. If statistical differences were found, Bonferroni-corrected comparisons with baseline measurements were performed. Regression analysis (linear and polynomial) was used to calculate coefficient of determination ( $r^2$ ) as a general summary statistic for describing the concurrency between the mean values of SAP<sub>LF</sub> and MSNA measures. Additionally, coefficients of determination were determined for individual data points from all subjects at baseline as well as for responses elicited by LBNP within each individual subject. All data are presented as means ± SEM unless otherwise specified and exact  $P$  values are presented for all comparisons.

### Results

Resting sympathetic activation levels varied greatly among subjects, whether assessed by burst frequency

(range: 4.3–35.7 bursts min<sup>-1</sup>) or total MSNA (range: 13–107 au). At baseline, low frequency oscillations in SAP (SAP<sub>LF</sub>) were not associated with MSNA burst frequency ( $r^2 = 0.02$ ), total MSNA ( $r^2 = 0.02$ ) or low frequency oscillations in MSNA (MSNA<sub>LF</sub>;  $r^2 = 0.04$ ). Similarly, low frequency oscillations in DAP (DAP<sub>LF</sub>) were not associated with MSNA parameters ( $r^2 \leq 0.13$ ). Furthermore, the coherence between DAP and MSNA was  $\geq 0.5$  in only 9 of our 20 subjects (45%) at baseline; similarly, the coherence between SAP and MSNA was  $\geq 0.5$  in 10 of 20 subjects (50%).

Average time to presyncope was  $1774 \pm 110$  s. Presyncope occurred in two subjects at –30, three at –60, six at –70, three at –80, five at –90 and one at –100 mmHg. Haemodynamic, respiratory and oscillatory responses during LBNP are shown in Table 1. Stroke volume and SAP decreased progressively during LBNP, while DAP and respiratory rate were not significantly altered. Heart rate increased during LBNP. High frequency oscillations in both SAP and DAP increased, but only at high levels of LBNP ( $\geq 60$  mmHg). Coherence between MSNA and both SAP and DAP was maintained at or above baseline levels throughout LBNP.

Figure 1 depicts blood pressure and MSNA responses in a representative subject during progressive central hypovolaemia induced by LBNP. Reductions in stroke volume during LBNP (Table 1) were associated with increases in MSNA that were evident early in the course of LBNP (i.e. at –30 mmHg). By –60 mmHg, both MSNA and blood pressure demonstrated an increase in oscillatory patterns that became more pronounced at –80 mmHg (Fig. 1). Group averages for MSNA burst frequency, total MSNA, low frequency oscillations in SAP (SAP<sub>LF</sub>) and MSNA (MSNA<sub>LF</sub>) during LBNP are presented in Fig. 2. MSNA increased progressively during LBNP, with a return to



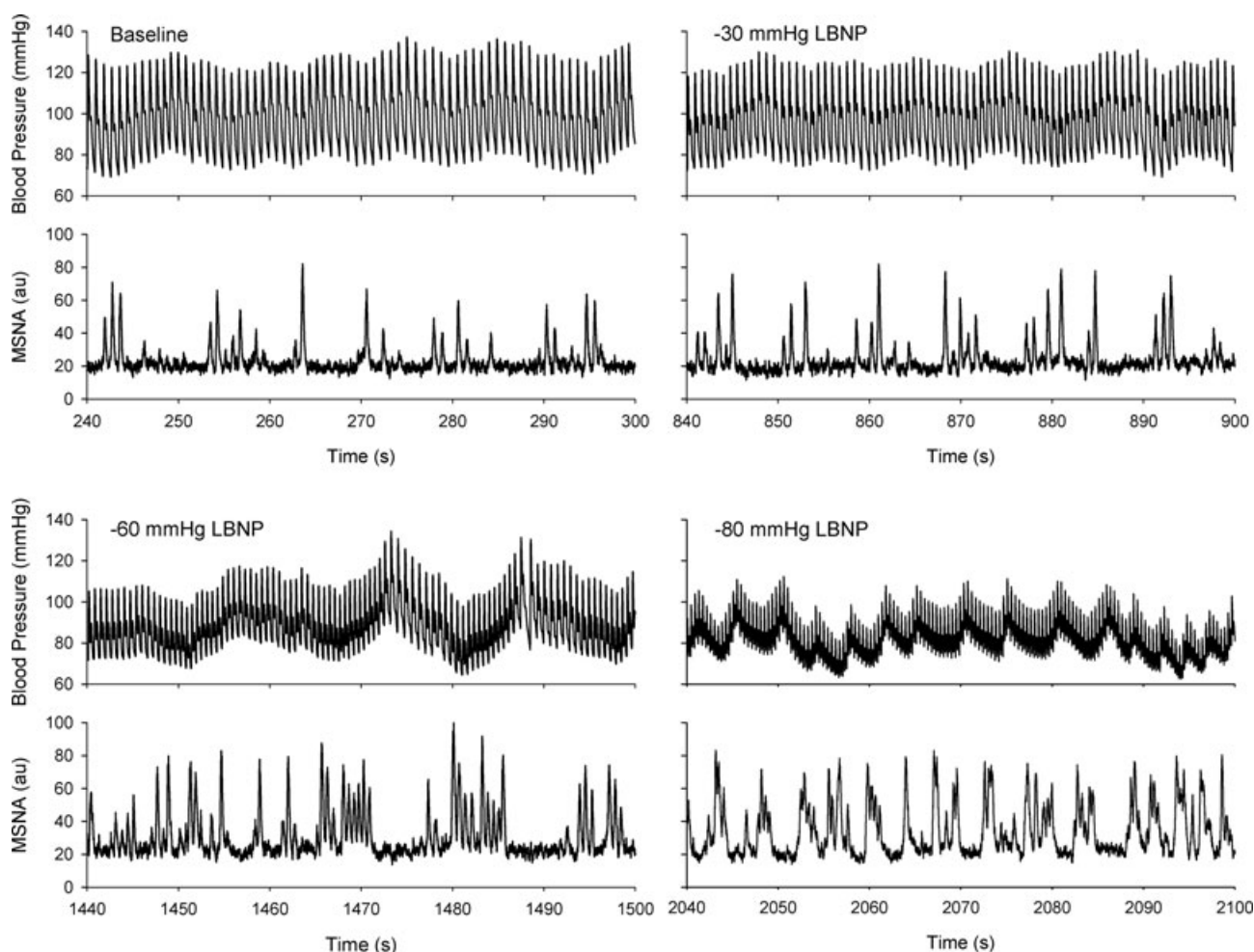
baseline levels upon cessation of LBNP. For the entire group of subjects,  $SAP_{LF}$  and  $MSNA_{LF}$  increased from baseline only at high levels of LBNP ( $\geq 60$  mmHg).

Relationships between mean values of  $SAP_{LF}$  and MSNA parameters are shown in Fig. 3. Despite increases in sympathetic activation during even early levels of LBNP,  $SAP_{LF}$  did not increase initially. Because of this, the association between  $SAP_{LF}$  and direct measurements of MSNA (either MSNA frequency or total MSNA) were curvilinear rather than linear. Coefficients of determination ( $r^2$ ) assuming linear relationships were 0.73 and 0.84 for MSNA frequency and total MSNA, but rose to 0.89 and 0.98 when quadratic functions were used. In contrast, the relationship between  $SAP_{LF}$  and  $MSNA_{LF}$  was highly linear ( $r^2 = 0.97$ ).

While group mean data demonstrated a strong curvilinear relationship between  $SAP_{LF}$  and MSNA, plotting of the association between these variables during LBNP for each individual subject revealed a great

deal of inter-individual variability (Fig. 4). Furthermore, coefficients of determination ( $r^2$ ) describing relationships between  $SAP_{LF}$  and MSNA within each subject varied widely between subjects, whether calculated using linear or quadratic equations (Table 2). Indeed, 7 of 20 subjects (35%) demonstrated  $r^2 < 0.5$  when linear regression was applied and 4 of 18 subjects (22%) demonstrated  $r^2 < 0.5$  when quadratic equations were used (data from the 2 subjects who experienced presyncope at  $-30$  mmHg could not be used because they had too few data points for valid polynomial regression analysis). Likewise, high individual variability characterised the relationship between  $SAP_{LF}$  and  $MSNA_{LF}$  produced by LBNP (Table 2); 30% and 22% of subjects showed  $r^2 < 0.5$  using linear and quadratic regression analysis.

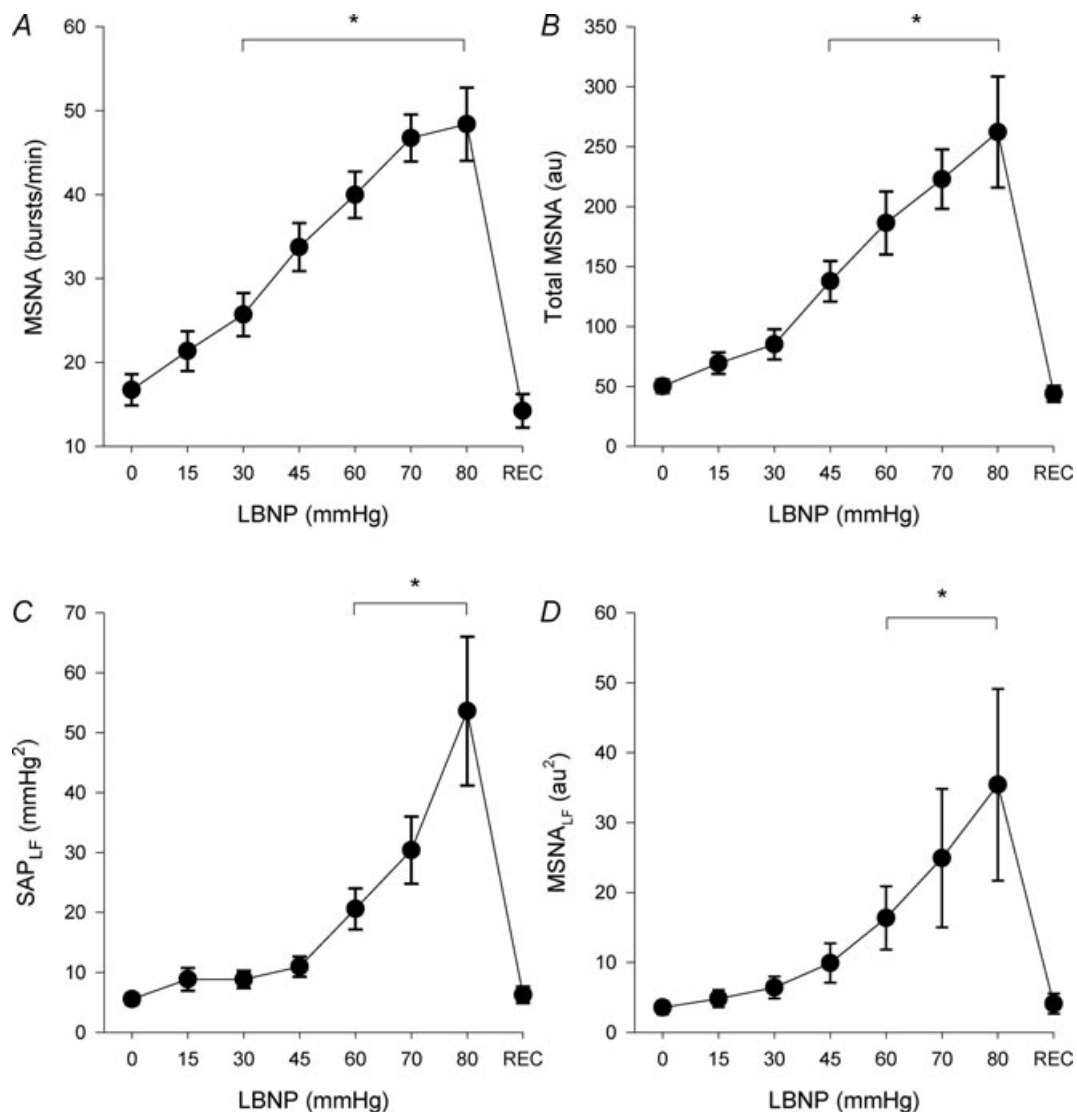
Because of the wide range of LBNP tolerances in our subjects, we subsequently divided subjects into either high tolerant (those who completed at least the  $-60$  mmHg level of LBNP;  $n = 15$ ) or low tolerant (those who



**Figure 1.** Blood pressure and muscle sympathetic nerve activity (MSNA) in a representative subject during baseline and progressive lower body negative pressure ( $-30$ ,  $-60$ ,  $-80$  mmHg LBNP)

did not complete the  $-60$  mmHg level;  $n=5$ ) groups. The high level of individual variability in linear  $r^2$  values persisted in both high tolerant (burst frequency,  $0.08\text{--}0.98$ ; total MSNA,  $0.12\text{--}0.94$ ;  $\text{MSNA}_{\text{LF}}$ ,  $7.87 \times 10^{-6}\text{--}0.99$ ) and low tolerant (burst frequency,  $1.65 \times 10^{-6}\text{--}0.83$ ; total MSNA,  $8.00 \times 10^{-4}\text{--}0.70$ ;  $\text{MSNA}_{\text{LF}}$ ,  $0.04\text{--}0.98$ ) groups. Polynomial regression produced similar variability in  $r^2$  values in high tolerant (burst frequency,  $0.09\text{--}0.99$ ; total MSNA,  $0.09\text{--}0.96$ ;  $\text{MSNA}_{\text{LF}}$ ,  $0.30\text{--}1.0$ ) and low tolerant (burst frequency,  $0.16 \times 10^{-6}\text{--}0.94$ ; total MSNA,  $0.09\text{--}0.90$ ;  $\text{MSNA}_{\text{LF}}$ ,  $0.06\text{--}0.63$ ) groups.

Low frequency oscillations in diastolic arterial pressure ( $\text{DAP}_{\text{LF}}$ ) also increased during LBNP (Table 1). Linear relationships between mean values of  $\text{DAP}_{\text{LF}}$  and MSNA parameters were described by higher coefficients of determination ( $0.83$  for burst frequency and  $0.91$  for total MSNA) than those of  $\text{SAP}_{\text{LF}}$  and MSNA, although quadratic fits of the data were even better ( $0.94$  for burst frequency and  $0.99$  for total MSNA). As with  $\text{SAP}_{\text{LF}}$ , the relationship between mean values of low frequency oscillations in both DAP and MSNA were highly linear ( $r^2 = 0.99$ ). However, interrogation of relationships between  $\text{DAP}_{\text{LF}}$  and MSNA parameters within each



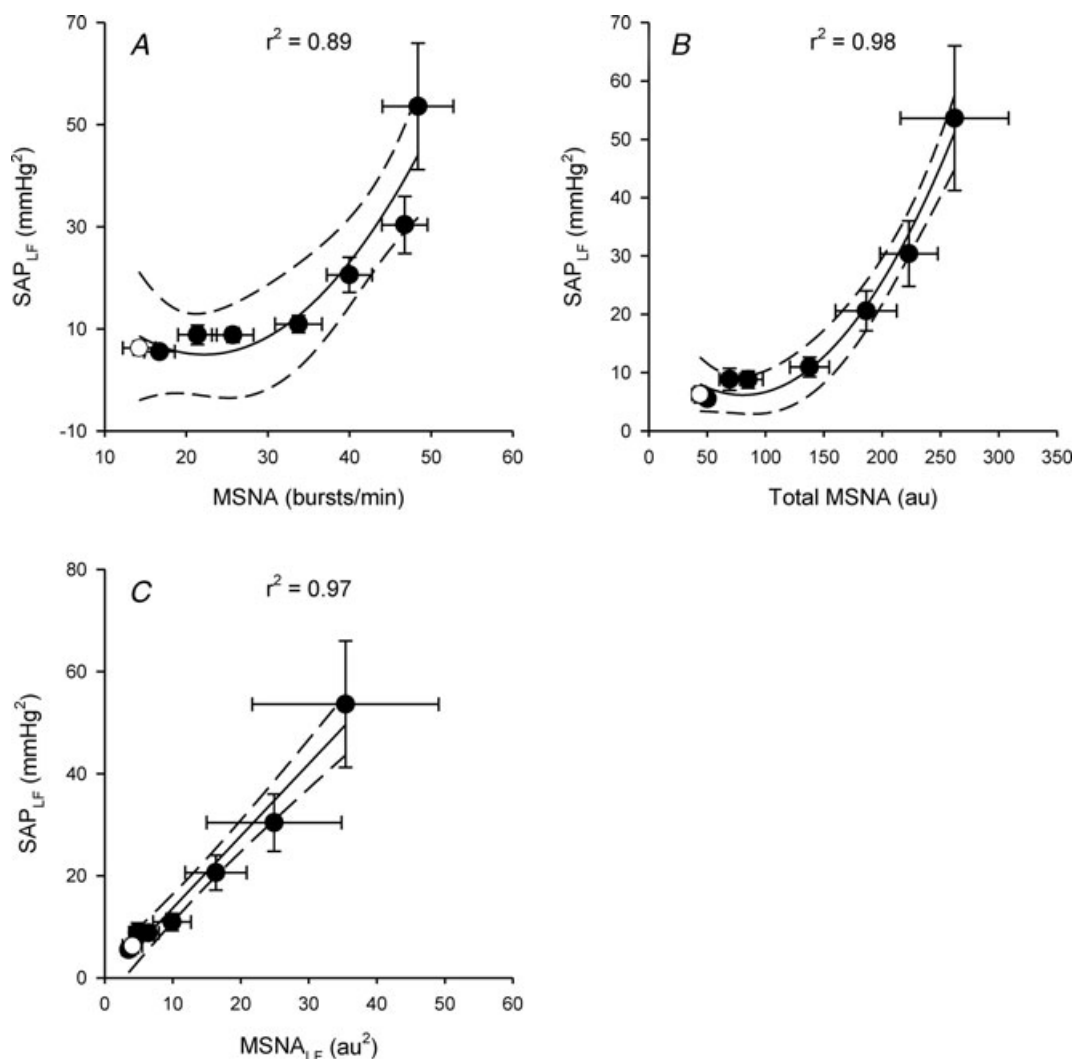
**Figure 2.** Muscle sympathetic nerve activity (MSNA; **A** and **B**), low frequency oscillations in systolic arterial pressure ( $\text{SAP}_{\text{LF}}$ ; **C**), and low frequency oscillations in MSNA ( $\text{MSNA}_{\text{LF}}$ ; **D**) during progressive lower body negative pressure (LBNP) and recovery (REC)

\*For all points included within bars,  $P \leq 0.021$  compared with baseline (0 mmHg LBNP) level. Sample size ( $n$ ) = 20 at 0, 15 and 30 mmHg LBNP and REC;  $n = 18$  at 45 mmHg LBNP;  $n = 17$  at 60 mmHg LBNP;  $n = 13$  at 70 mmHg LBNP;  $n = 7$  at 80 mmHg LBNP.

individual subject again revealed very large ranges of  $r^2$  values, whether linear or polynomial regressions were used (Table 3). Just as with  $SAP_{LF}$ , 7 of 20 subjects (35%) demonstrated  $r^2 < 0.5$  when linear regression was applied to relationships between  $DAP_{LF}$  and MSNA parameters and 4 of 18 subjects (22%) demonstrated  $r^2 < 0.5$  when quadratic equations were used. For the relationship between  $DAP_{LF}$  and  $MSNA_{LF}$ , 35% and 28% of subjects demonstrated  $r^2 < 0.5$  using linear and polynomial (quadratic) regression analysis. Inter-individual variability was not dependent on the ability to tolerate LBNP (data not shown).

## Discussion

There are two primary findings from this study. First, when considering the population as a whole, the relationship between  $SAP_{LF}$  and direct measurement of sympathetic nerve activity is curvilinear rather than linear during progressive central hypovolaemia. More importantly, responses in  $SAP_{LF}$  to increasing sympathetic activation are highly variable among individuals. These results indicate that  $SAP_{LF}$  cannot be used universally as a non-invasive metric of sympathetic outflow in individual subjects in either the resting condition or during sympathetic activation.



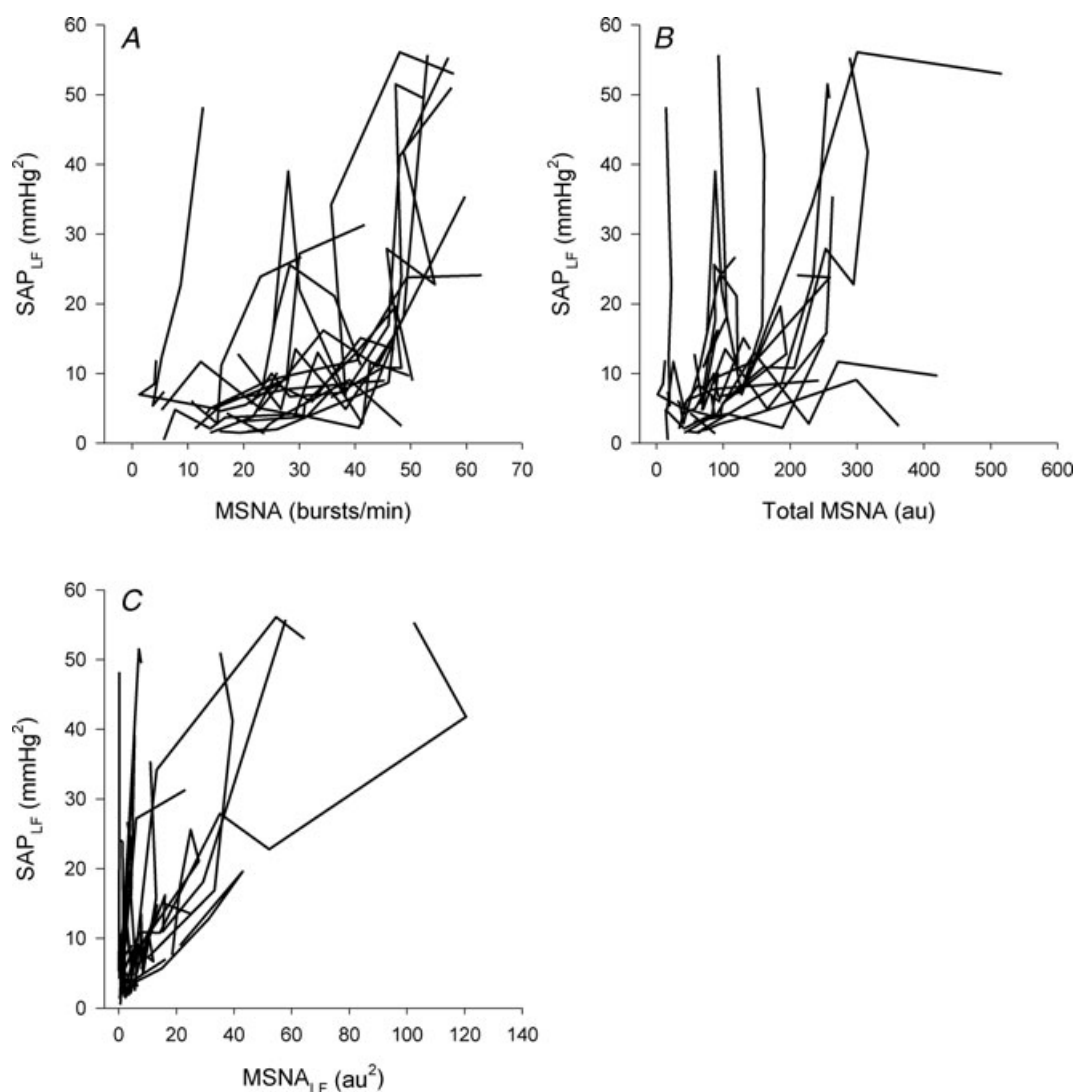
**Figure 3. Associations between low frequency oscillations in systolic arterial pressure ( $SAP_{LF}$ ) and muscle sympathetic nerve activity (MSNA; A and B) and low frequency oscillations in MSNA ( $MSNA_{LF}$ ; C) during progressive lower body negative pressure**

Open circle denotes recovery point; continuous line represents best fit with 95% confidence intervals (dashed lines). Coefficients of determination ( $r^2$ ) reflect polynomial (A and B) or linear (C) regression on mean values. Sample size ( $n$ ) = 20 at 0, 15 and 30 mmHg LBNP and recovery;  $n$  = 18 at 45 mmHg LBNP;  $n$  = 17 at 60 mmHg LBNP;  $n$  = 13 at 70 mmHg LBNP;  $n$  = 7 at 80 mmHg LBNP.



Analysis using group means first demonstrated that the relationship between  $SAP_{LF}$  and MSNA during sympathetic activation was not a direct linear relationship but was instead curvilinear. This was not unexpected, as we had previously observed that low frequency oscillations in arterial pressure increase significantly only at high levels of LBNP (Cooke *et al.* 2009) while MSNA burst frequency and total MSNA increased at lower levels (Cooke *et al.* 2009). Furlan *et al.* (2000), however, had previously shown that orthostatic stress induced by progressive head up tilt produced a 2-fold increase in MSNA burst frequency but a 12-fold increase in  $SAP_{LF}$ . This profound increase in  $SAP_{LF}$  occurred despite only a doubling of  $MSNA_{LF}$  (Furlan *et al.* 2000). In the present data set, however, MSNA was approximately doubled at  $-45$  mmHg LBNP and was accompanied by doubling of both  $SAP_{LF}$  and

$MSNA_{LF}$  (Fig. 2); in fact, group means of  $SAP_{LF}$  were highly correlated ( $r^2 = 0.97$ ) with  $MSNA_{LF}$  at all levels of sympathetic activation throughout LBNP (Fig. 3). Despite these quantitative differences in  $SAP_{LF}$ , our results are consistent with the conclusion reached by Furlan *et al.* (2000) that, when group mean values are considered, sympathoexcitation increases the coupling between low frequency spectral components of MSNA and SAP. While our group mean results suggest that  $SAP_{LF}$  might be used as a non-invasive surrogate of  $MSNA_{LF}$ , it is clear that  $SAP_{LF}$  is not linearly related to MSNA and therefore should not be used in this fashion as a non-invasive surrogate for MSNA. This conclusion is in agreement with the results from numerous animal studies (Persson *et al.* 1992; Malpas, 1995; Stauss *et al.* 1995; Tsai *et al.* 2009).



**Figure 4.** Individual subject associations between low frequency oscillations in systolic arterial pressure ( $SAP_{LF}$ ) and muscle sympathetic nerve activity (MSNA; A and B) and low frequency oscillations in MSNA ( $MSNA_{LF}$ ; C) during progressive lower body negative pressure  $n = 20$ .

**Table 2. Coefficients of determination ( $r^2$ ) for associations between low frequency oscillations in systolic arterial pressure ( $SAP_{LF}$ ) and muscle sympathetic nerve activity (MSNA) and its oscillations ( $MSNA_{LF}$ ) during LBNP for each individual subject**

	Mean $\pm$ SEM	Range
Linear fit ( $n = 20$ )		
MSNA ( $b \min^{-1}$ ) vs. $SAP_{LF}$	$0.52 \pm 0.07$	$1.62 \times 10^{-6}$ –0.98
Total MSNA (au) vs. $SAP_{LF}$	$0.54 \pm 0.07$	$8.03 \times 10^{-4}$ –0.94
$MSNA_{LF}$ vs. $SAP_{LF}$	$0.63 \pm 0.07$	$7.87 \times 10^{-6}$ –0.99
Quadratic Fit ( $n = 18$ )		
MSNA ( $b \min^{-1}$ ) vs. $SAP_{LF}$	$0.67 \pm 0.06$	0.09–0.99
Total MSNA (au) vs. $SAP_{LF}$	$0.67 \pm 0.07$	0.09–0.96
$MSNA_{LF}$ vs. $SAP_{LF}$	$0.72 \pm 0.07$	0.06–1.0

**Table 3. Coefficients of determination ( $r^2$ ) for associations between low frequency oscillations in diastolic arterial pressure ( $DAP_{LF}$ ) and muscle sympathetic nerve activity (MSNA) and its oscillations ( $MSNA_{LF}$ ) during LBNP for each individual subject**

	Mean $\pm$ SEM	Range
Linear Fit ( $n = 20$ )		
MSNA ( $b \min^{-1}$ ) vs. $DAP_{LF}$	$0.55 \pm 0.06$	$4.93 \times 10^{-3}$ –0.95
Total MSNA (au) vs. $DAP_{LF}$	$0.58 \pm 0.07$	$1.28 \times 10^{-3}$ –0.97
$MSNA_{LF}$ vs. $DAP_{LF}$	$0.68 \pm 0.07$	$1.31 \times 10^{-4}$ –1.0
Quadratic Fit ( $n = 18$ )		
MSNA ( $b \min^{-1}$ ) vs. $DAP_{LF}$	$0.67 \pm 0.06$	0.22–0.98
Total MSNA (au) vs. $DAP_{LF}$	$0.70 \pm 0.07$	$7.21 \times 10^{-3}$ –1.0
$MSNA_{LF}$ vs. $DAP_{LF}$	$0.75 \pm 0.06$	0.30–1.0

The existence of a curvilinear relationship between  $SAP_{LF}$  and MSNA could, however, be useful as a non-invasive surrogate of sympathetic nerve activity as long as (1) the curvilinearity of the relationship is recognized and appropriate equations are used; and (2) this relationship is consistently strong among individual subjects. We therefore sought to determine whether  $SAP_{LF}$  reliably tracked MSNA metrics on an individual subject basis. In our 20 subjects at baseline, neither MSNA nor  $MSNA_{LF}$  was correlated with  $SAP_{LF}$ . Additionally, coherence values were  $<0.5$  in approximately 50% of our subjects, indicating a lack of concordance between the oscillatory patterns in  $SAP_{LF}$  and  $MSNA_{LF}$  in many subjects. These results confirm those of Taylor *et al.* (1998) acquired during the resting state in healthy subjects of differing sexes and ages and therefore different baseline levels of sympathetic activation. Our study extends this previous work by demonstrating very high inter-individual variability in the relationships between  $SAP_{LF}$  and MSNA during profound sympathetic activation elicited by LBNP. This high level of inter-individual variability persisted with linear and polynomial analyses and was not dependent on the ability of the individual to tolerate LBNP. Importantly,  $r^2$  values characterising

the associations between these variables were  $<0.5$  in 22% of our subjects even when the curvilinear nature of the association was taken into account. It is therefore clear that the use of  $SAP_{LF}$  to indicate the level of sympathetic activation is not valid in all subjects. Because of the wide inter-individual variability and the very weak  $SAP_{LF}$ –MSNA association in a substantial proportion of the population, the use of  $SAP_{LF}$  as a non-invasive surrogate of MSNA is not reliable and therefore not warranted.

These results are in contrast to the conclusion drawn by Pagani *et al.* (1997), who used infusions of sodium nitroprusside and phenylephrine to induce sympathoexcitation and sympathoinhibition. When plotted as individual points, these investigators demonstrated highly significant  $P$  values for correlations between  $SAP_{LF}$  and MSNA metrics, but did not provide correlation coefficients (Pagani *et al.* 1997). Taylor *et al.* subsequently used the reported data of Pagani *et al.* to calculate correlation coefficients ( $r$ ) of  $\leq 0.46$  for the relationships between  $SAP_{LF}$  and MSNA (Taylor *et al.* 1998). The results from our study eliciting sympathoexcitation using nonpharmacological means suggest that the weak association between these variables in the previous study may reflect profound individual variability in the response of  $SAP_{LF}$  to sympathetic activation, with some subjects demonstrating strong correlations and others demonstrating remarkably weak correlations. Taken together, these results indicate that the use of  $SAP_{LF}$  as a non-invasive surrogate of MSNA either at rest or during sympathetic activation cannot be justified in individual subjects. Assessment of autonomic function using  $SAP_{LF}$  during a clinical test involving sympathoexcitation therefore may not be appropriate, as even healthy individuals do not reliably demonstrate associations between  $SAP_{LF}$  and direct measurement of sympathetic nerve activity.

Although our main intent was to investigate the proposed use of  $SAP_{LF}$  as a non-invasive surrogate of MSNA, diastolic pressure is more closely related to sympathetic nerve activity than systolic pressure (Sundlof & Wallin, 1978). Indeed, relationships between  $DAP_{LF}$  and MSNA parameters demonstrated a higher degree of linearity than did  $SAP_{LF}$  in our study. However, further analysis revealed extremely high variability in the strength of the relationships between  $DAP_{LF}$  and MSNA among individual subjects, just as in the associations between  $SAP_{LF}$  and MSNA. Hence, low frequency oscillations in arterial blood pressure, whether systolic or diastolic, cannot be used as non-invasive surrogates of sympathetic nerve activity.

The lack of reliable associations between low frequency oscillations in blood pressure and MSNA should not be surprising. Low frequency oscillations in arterial pressure have been suggested to be produced by either the action of

central oscillating pacemaker neurons or by a resonance in the baroreflex (Julien, 2006). In either case, low frequency rhythms of sympathetic nerve activity would be produced via central mechanisms, and it is these rhythms rather than an absolute level of sympathetic activation that act to produce low frequency oscillations in blood pressure (Malpas, 1998). The observation that heat stress produces profound sympathetic activation without an increase in  $SAP_{LF}$  can therefore be understood because  $MSNA_{LF}$  did not concomitantly increase (Cui *et al.* 2004). However, it would be simplistic to assume that oscillations in sympathetic nerve activity would be directly reflected as a proportional oscillation in blood pressure, as the ability of the nerve signal to be transduced into vascular resistance (Myers *et al.* 2001) and intrinsic vasomotor rhythmicity of blood vessels (Stauss, 2007) must also contribute to the genesis of blood pressure oscillations. Indeed, low frequency oscillations in blood pressure persist, albeit at lower levels, following complete ganglionic blockade in humans (Zhang *et al.* 2002). Furthermore, O'Leary *et al.* (2004) demonstrated in an elegant study that the increased blood pressure variability associated with head up tilt was the result of both baroreflex regulation through the sympathetic nervous system and a non-baroreflex component presumed to be a myogenic response. It is possible that summation of myogenic responses with input from sympathetic nerves at the level of the vasculature might underlie the lack of strong relationships between  $MSNA_{LF}$  and  $SAP_{LF}$  in individual subjects either at baseline or during sympathetic activation. Our results therefore provide an additional caution against the use of  $SAP_{LF}$  to provide information about  $MSNA_{LF}$  in individual subjects.

While the use of group mean data for insight into physiological processes is necessary and appropriate, further investigation is required before advocating the use of a non-invasive surrogate measure in clinical settings. Such non-invasive measurements as heart rate variability have been suggested for use in tracking compensatory autonomic responses to physiological challenges based on group mean data, but have failed to provide reliable information in individual subjects (Rickards *et al.* 2010; Ryan *et al.* 2010). In this study, we applied the same 'next step' approach to the data presented herein, moving from the group mean level to the individual level. With this approach, we determined that neither  $SAP_{LF}$  nor  $DAP_{LF}$  can be reliably used as a non-invasive surrogate of  $MSNA$  in individuals, despite its convenience of use. We therefore strongly advocate the use of this 'next step' approach for investigation of non-invasive surrogates to ensure clinical utility in individual patients.

This study is not without potential methodological limitations. First, arterial blood pressure oscillations were determined by the use of non-invasive photoplethysmography rather than via a direct arterial catheter.

However, measurement of blood pressure by finger photoplethysmography has been shown to be highly accurate compared with direct arterial measurement during a variety of physiological manoeuvres including orthostatic stress (Imholz *et al.* 1988; Parati *et al.* 1989; Imholz *et al.* 1990). More germane to this study, use of the Finometer technology has been validated against direct intra-arterial recordings of blood pressure for use in power spectral analysis of blood pressure variability (Omboni *et al.* 1993). While it is recognized that blood pressure measurement using this technique may be affected when temperature of the finger or body is altered (Imholz *et al.* 1998), all experiments in this study were conducted within a small range of ambient temperature, suggesting little to no temperature-induced alteration in vasomotor tone during the experiments. Additionally, measurement of  $MSNA$  might not be reflective of sympathetic nerve activity to other vascular beds (Malpas, 2010). We therefore cannot discount the possibility that direct measurement of sympathetic nerve activity in other vascular beds might yield improved relationships with low frequency oscillations in arterial pressure.

Validation of a non-invasive metric that could be used as a surrogate for direct measurement of sympathetic nerve activity would be useful both for clinical investigation of dysautonomias as well as for laboratory investigations. Measurement of  $SAP_{LF}$  has been suggested as such a metric based primarily on group mean data contained within earlier studies. While curvilinear relationships exist between  $SAP_{LF}$  or  $DAP_{LF}$  and  $MSNA$  on a group level, neither  $SAP_{LF}$  nor  $DAP_{LF}$  is reliably correlated with absolute levels of  $MSNA$  either at rest or during central hypovolaemia in individual subjects. These data therefore do not support the use of low frequency oscillations in arterial pressure as a non-invasive surrogate of  $MSNA$ .

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### Author contributions

All experiments were performed in the laboratory of the Advanced Capabilities for Emergency Medical Monitoring Task Area at the US Army Institute of Surgical Research, Fort Sam Houston, TX, USA. The authors, K.L.R., C.A.R., C.H.-L., W.H.C. and V.A.C. contributed to: conception and design of the experiments; collection, analysis and interpretation of data; and drafting the article and revising it critically for important intellectual content. All authors approved the final version of the manuscript.

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